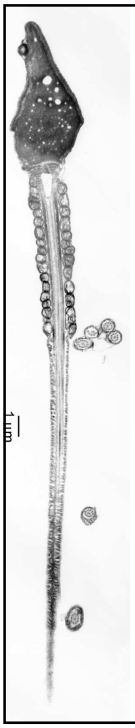
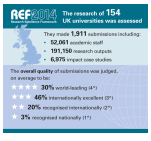
AT: What journals can do : In Press MHR
32 experts

MHR Basic science of reproductive medicine

THE WHY, THE HOW AND THE WHEN OF PGS2.0: CURRENT PRACTICES AND EXPERT OPINIONS OF FERTILITY SPECIALISTS, MOLECULAR BIOLOGISTS, AND EMBRYOLOGISTS



First for Biological Science in UK



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Declaration of Conflicts of Interest

- Fully employed by University of Dundee (UoD).
- WHO paid honorarium to be Chair of group and paid travel/accommodation/expenses.
- Editor in Chief of MHR (honorarium and expenses)
- Grant funding from MRC.
  - UoD Patent – sperm stimulation.
- Give occasional lectures that are company/society sponsored : pay travel/accommodation/expenses sometimes small honorarium.
- Cambridge University Press – 2 edited books.
- I'm not on any company board or have a single share in anything or anybody.

Background – some queries.

- In overwhelming majority of cases aneuploidy (autosomal) associated with female. But specific associations with male Helen this am : 15%, 80% 45X
- In the context of ART is there a higher risk and can this be managed?
- Focus on sperm - specific groups with increased risk:
  - Is the proportion of aneuploidy spermatozoa higher in sub fertile men? Yes but very variable data (50+studies).
  - Do sub fertile men normally present higher chance of aneuploidy in foetus/offspring e.g. ICSI (Bonduelle et al., examples) translation example PGD ESHRE data.
  - Recent evidence for a paternal contribution to aneuploidy in human embryos (CCS).
- Interesting examples (47XXY; Globozoospermia; very specific sperm abnormalities; Trisomy 21).
- What can be done ? (apart from PGD)

Example review : Templado et al., 2013 MHR 19, 634-643. Harton & Tempest 2012 AJA 14, 32.

Sub-fertile men (?severely) men normally give higher chance of aneuploidy sperm.

- Considerable degree of evidence – in sub fertile men more severe with increasing severity of male infertility.
- RPL: Zidi Jrah et al., (2016) higher level sperm aneuploidy in partners of women with recurrent pregnancy loss cf., controls
- Ramasamy et al (2015) n= 140 men who's partners had recurrent pregnancy loss -
  - greater % of sperm aneuploidy with sex chromosomes and 18 and 13/21 vs controls (1.04% vs 0.38%; 00.18 vs 0.03; 0.26 vs 0.08) and demonstrating that 40% men with RPl had significantly higher sperm aneuploidy (and greater in men with lower semen characteristics).

Table II Levels of aneuploidy in sperm of selected male populations[a].

Males population with		Sex chr. disomy	Chr. 21 disomy	Total disomy	Conservative aneuploidy	Diploidy
Stable variants	Rubes *et al.* (2002, 2005)	3x				5x
Aneuploid offspring	Reviewed in Templado *et al.* (2011)	2x	2x			
Repeated spontaneous abortions	Rubio *et al.* (1999)	2.3x				
Infertility	Sarrate *et al.* (2010)	2–3x	2–3x		3x	3–5x
Severe non-obstrucive oligozoospermia	Mougou-Zerelli *et al.* (2011)	4x				2x
Severe oligozoospermia	Durak Aras *et al.* (2012)	2–6x	4x			
Non-obstructive azoospermia	Sun *et al.* (2008)	2–4x	4x			
Polymorphic teratozoospermia	Brahem *et al.* (2011a) Templado *et al.* (2002)	 2x		4–10x	 2–3x	2.5x
Unclassified teratozoospermia	Tang *et al.* (2010) Gole *et al.* (2001)	1.5x 4x	3x		2.5x	2x
Globozoospermia	Brahem *et al.* (2011a) Morel *et al.* (2004)	 2–3x		8–10x		4x
Macrocephalic head syndrome	Brahem *et al.* (2011a) Brahem *et al.* (2011b)			10–30x		23% 22%

[a]Expressed as an order of magnitude compared with internal controls of each study.

Templado et al., 2013 MHR 19, 634-643.

Aneuploidy in embryos
Related to severe male sub fertility ?

Specific studies : Paternal contribution to aneuploidy in embryos

Retrospective cohort study 3835 embryos from 629 couples

Aneuploidy in trophectoderm from IVF and ICSI (normal and oligo) (CCS)

Patient data followed by collective and individual levels of aneuploidy (including percentages) for each patient group.

	Group using their own eggs			Group using donor eggs			Total number	*P* value
Variable	**Standard IVF**	**ICSI, normal sperm**	**ICSI oligozoospermia**	**Standard IVF**	**ICSI, normal sperm**	**ICSI oligozoospermia**		
Mean maternal age (y)	35.5	35.3	35.3	24.9	25.0	25.0		
No. of cycles	77	262	31	25	222	12	629	
No. of embryos	385	1,300	114	208	1,743	85	3,835	
Embryos for biopsy, mean	5	5	3.7	8	7.9	7.1		
Total aneuploidy (%/embryo)	158 (41)	477 (37)	53 (46)	44 (21)	394 (23)	23 (27)	1,307	NS
Total with autosomal aneuploidy (%/embryo)	155 (40)	466 (39)	51 (45)	42 (20)	384 (22)	19 (22)	1,117	NS
Total (%) with sex chromosome aneuploidy	8 (2.1)	22 (1.6)	7 (6.1)*	3 (1.4)	35 (2.0)	5 (5.9)**	40	*0.006 **0.04
Individual aneuploidies	XXX XO ×3 XXY ×3 XYY	XO ×12 OY XXYY XXX ×4 XXY ×2 XYY ×2	XO ×5 XXY XXX	XO ×2 XXY	XO ×20 OY ×2 XYY ×2 XXY ×9 XXX ×2	XO ×2 XXY ×2 OY		

Coates et al. 2015, Fertil Steril 104, 866

Summary/discussion from Coates et al.

- As examined IVF and ICSI indicated ICSI procedure itself not a significant variable.
- Maternal age not a variable as same in donor vs own egg group.
- Oligozoospermia significantly associated with aneuploidy in embryos by implication paternal. As 47 XXX (5% paternal), 47 XXY (50%), 45XO(~75%), 47 XYY (100%) - if paternal effect in study would see fewer 47XXX and 450Y. This is case (10/12 not these)
- However parent (and phase) of origin analysis is required.

KS (XXY) syndrome

- Common disorder ~ 1:600 males ; ~10% have mosaic

EAU Guidelines

(Jungwirth et al., 2012 Eur Urol. 62:324)
Significant contribution from XXY

Diagnosis	Unselected 12,945	Azoospermic 1446
Idiopathic	30.3 %	13.3%
KS (XXY)	2.6%	13.7%
Y deletions	0.3%	1.6%

Significant component unknown.

ART in Non Mosaic KS

Sperm in the ejaculate have been found in up to 8% of non mosaic men.

Produce higher levels of aneuploidy spermatozoa (sex chromosomes and some autosomes [13/18/21]) (50-93% cells normal but majority show +90% normal

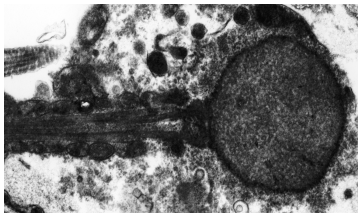
Rare natural conceptions published first ICSI 1997

Consistent pregnancies at ART at least 180 children born overwhelming majority (all) normal karyotype.

PGD indicated higher aneuploidy rate in embryos (Staessen et al., 2003) particular sex chromosome and 18/21 but 54% normal (n=113 embryos; 13% vs 3% sex chromosome; 15 vs 5% autosomal cf controls) but no XXY).

Van Saen et al., (2012) Fertil Steril , 97, 319; (Bourne et al., Hum Reprod 12, 2447-2450). Aksglaede & Juul EJ Endo (2013) 168, R67. Rives et al., 2000 MHR 6, 107.

Globozoospermia –Sperm aneuploidy variable



- Incidence of globozoospermia 1:???????? sub-fertile men.
- Generally limited data as few cases reports

While plethora of scientific queries ..

- Meiotic competence and fidelity that results in aneuploidy e.g. KS
- What account for variability and subsequent effectiveness of function
- …

So what can we do (except PGD) Selecting the optimal cell

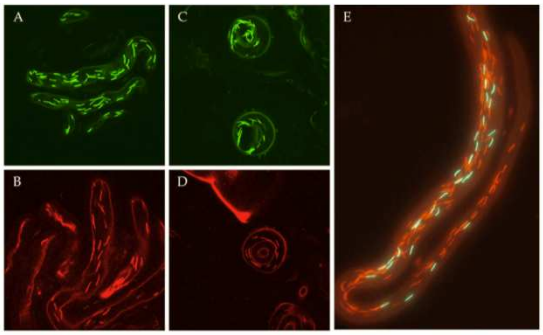
- In vivo ? And lessons
- In vitro : favourite protein/concept/idea.

Mate (gamete) selection in animals

Very sophisticated with somewhat of an evolutionary arms race pre and post copulation.

All aimed at selection of the fittest sperm cell

Dramatic and fierce competition once in female tract : selection by sperm and female

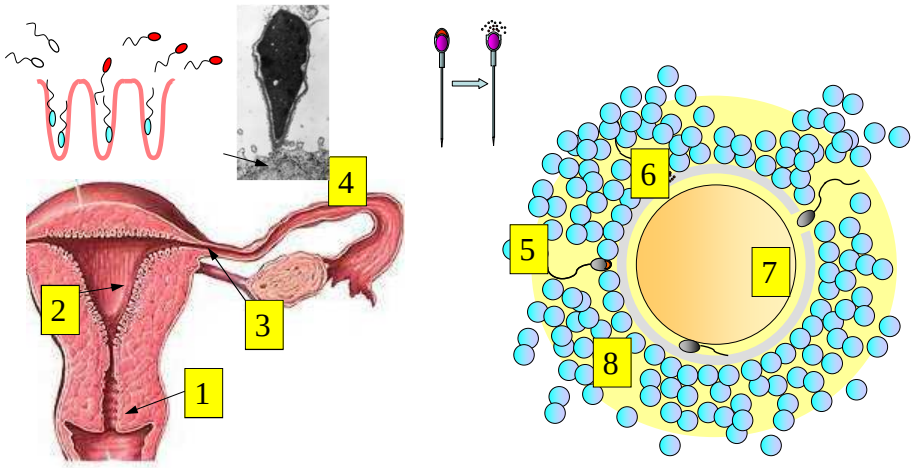


Removal of rival cell
Induction of response in female tract -gene expression

From Manier et al., 2010 Science 328:354-7.

The incredible journey – (presumably) selection of the best cells

1:14,000,000 reach site of fertilisation



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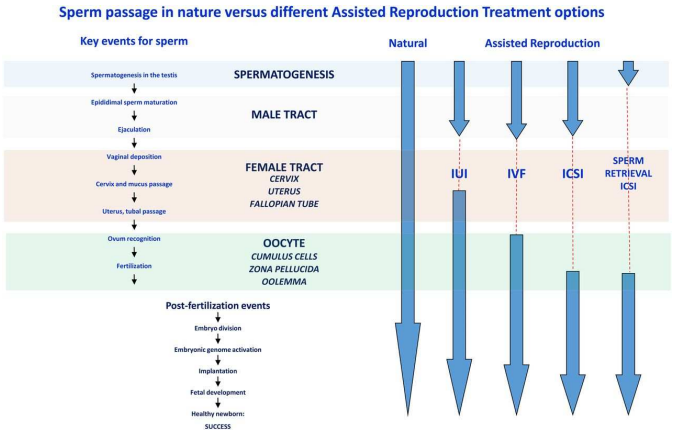
Adapted from Publicover, Harper & Barratt 2007 Nature Cell Biology 9, 235-42

BUT : Chromosomally abnormal spermatozoa are effective at fertilisation
and ???????possible not disadvantaged in natural/IVF fertilisation?

- Detailed experiments not done comparing effectiveness at fertilisation however,
- Paternal cases : KS, Trisomy 21 etc.
- Structural abnormalities (e.g. translocations [balanced]) (Thomas et al., 2010 J Med Genet. 47, 112).
- Maybe learn from female tract however what about ART?

ART ? Less rigorous selection



Denny Sakkas et al. Hum. Reprod. Update 2015;21:711-726

human reproduction update

Can ART sperm selection techniques reduce impact ?

Potential reduction in frequency of aneuploidy sperm bound to HA.

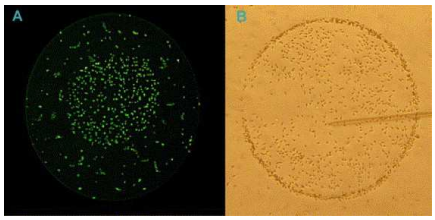
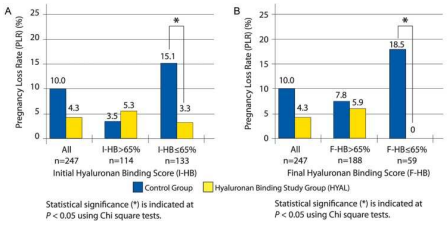


FIGURE 1. (A) Sperm approach from the periphery and then bind to the HA-spot. (B) Sperm being picked up with the ICSI pipette.Jakab. A novel method for ICSI sperm selection. Fertil Steril 2005.



Worrilow et al., Hum Reprod. 2013 28:306

Therefore potential to select better cells and reduce pregnancy loss in ART.

Potential techniques to select optimal cells
However..

- Current evidence was insufficient to permit evaluation of the effectiveness of advanced sperm selection strategies in assisted reproductive technologist (ART). No evidence showed a difference between the groups in terms of any of the reported outcomes.
- Further studies of suitable quality are required before any of these advanced sperm selection techniques can be recommended for use in clinical practice
- Evidence is current to May 2014.
- HA test : HAB select : http://www.habselect.org.uk/

Advanced sperm selection techniques for assisted reproduction (Review)

McDowell S, Kroon B, Ford E, Hook Y, Ghujovsky D, Yazdani A

Other techniques – electric charge - still a prototype

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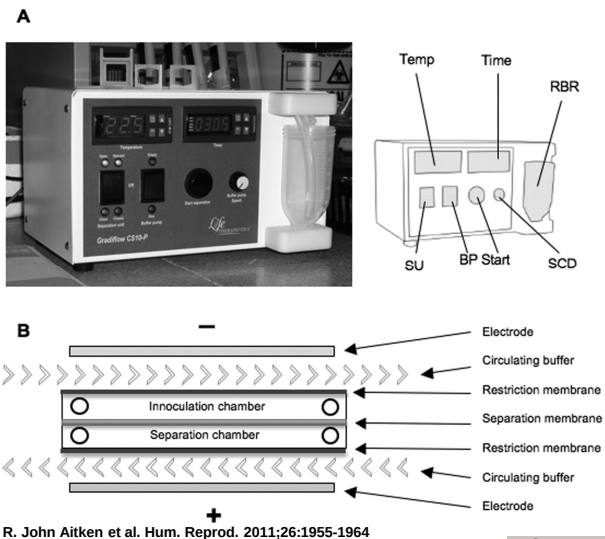


Additional systems :

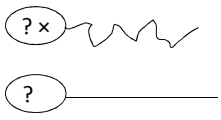
Simon et al., Fertil Steril. 2015 103:361

R. John Aitken et al. Hum. Reprod. 2011;26:1955-1964

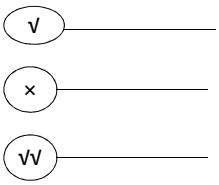
human reproduction

The fundamental truth

Current



Future



Poor quality cells=Reduced

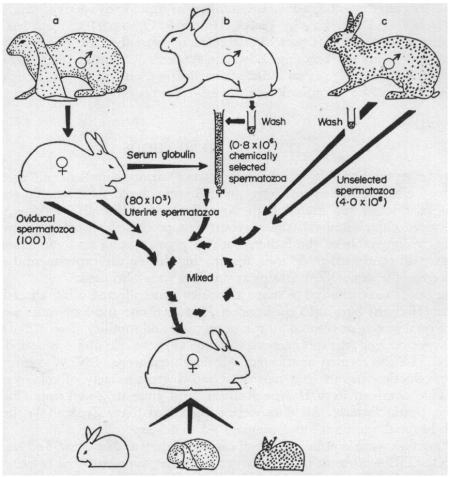
1. FR
2. Embryo dev
3. Implantation rate
4. CPR
5. **LBR**

Need to get the sperm to confess 'errors'

- Concept re examine *in vivo* interaction with new glasses
- 2 rather interesting examples...

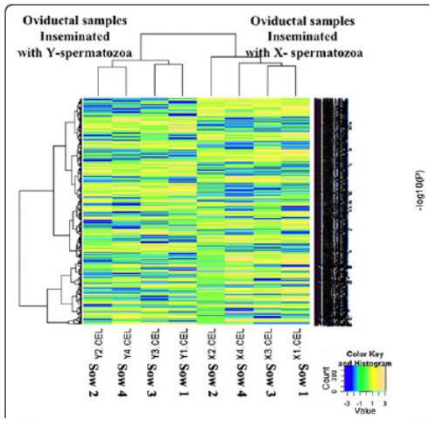
Sperm that reach the egg are (possibly) a selected population

(Cohen & McNaughton 1974, J Reprod Fertil 39, 297-310)



The female tract can detect and response to X vs Y sperm….

Alminana et al., BMC Genomics 2014 15, 293.



Summary

- Specific groups with increased risk:
  - Is the proportion of aneuploidy spermatozoa higher in sub fertile men? Yes but very variable data.
  - Do sub fertile men normally present higher chance of aneuploidy in foetus/offspring e.g. ICSI yes.
  - Recent evidence for a paternal contribution to aneuploidy in human embryos (CCS).
- Particular interesting examples (47XXY).
- Many scientific queries BUT.
- Female tract selection – possible lessons but yet to exploit these and perform detailed experiments.

Sarah, Steven M, Mythili, Mark, Wardah, Sean, Hannah, Clair, Ola, Vanessa

Collaborators in Dundee
All at IVF clinic for samples, patients
Paul Wyatt and Tony Hope [CLS]
Tim Newman [Engineering, Physics and Mathematics]

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Stuart Wilson [Durham]

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Timo [Munster]